

## ***In vitro* activity of capsaicin against *Helicobacter pylori***

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**Abstract** - *Helicobacter pylori* has increasingly become resistant to clarithromycin and metronidazole throughout the world. The aim of this study was to demonstrate the *in vitro* activity of capsaicin on metronidazole-susceptible and -resistant *H. pylori*. Sixteen clinic isolates (including eight metronidazole resistant strains) and references strain (NCTC 11637) of *H. pylori* were used in the study. Various concentrations of the capsaicin solution were tested. Capsaicin showed bactericidal effect even at the lowest prepared concentration (25 µg ml<sup>-1</sup>). The best effect was seen at concentration of 50 µg ml<sup>-1</sup> within 4 h of incubation. Active content of hot pepper, capsaicin, has *in vitro* activity against *H. pylori* and may be a useful alternative treatment strategy. This may provide a new and alternative treatment approach in eradication of metronidazole-resistant strains. The profound effect of capsaicin on *H. pylori* suggests that small doses of capsaicin can help in treatment of gastric and duodenum ulcer.

**Key words:** capsaicin, antimicrobial activity, *Helicobacter pylori*.

### **INTRODUCTION**

*Helicobacter pylori* is now accepted as a major cause of gastro-duodenal disease, gastric cancer, peptic and gastric ulcer. Additionally, clinical evidence shows that eradication of *H. pylori* results in significant remission from diseases (Graham, 1993; Malfertheiner *et al.*, 1997; Lam *et al.*, 1998; Wang *et al.*, 2002; Miwa *et al.*, 2004).

Capsaicin constitutes of pepper and gives its taste, also has pharmacological and physiological effects (Cordell *et al.*, 1993). It has also been shown that *Capsicum* species and also capsaicin have antimicrobial effects (Cichewicz *et al.*, 1996; Molina-Torres *et al.*, 1999). The treatment strategies used for *H. pylori* are not sufficient, new drugs and treatment strategies are needed. Capsaicin might be a possible alternative therapeutic agent for gastric diseases caused by *H. pylori*.

Jones *et al.* showed the effect of capsaicin on *H. pylori* (1997). However, the effect of capsaicin particularly on metronidazole-resistant *H. pylori* has not been studied yet.

Hot pepper is consumed as a flavouring spice especially in Southeast part of Turkey. Due to the high consumption rate of hot peppers in the area and anecdotal evidence of their benefits, we decided to investigate the effect of different concentrations of capsaicin on metronidazole-susceptible (MtzS) and -resistant (MtzR) *H. pylori in vitro*.

### **MATERIALS AND METHODS**

**Strains and growth condition** Sixteen clinical isolates of *H. pylori* (including eight MtzR strains) as well as *H. pylori* NCTC 11637 (National Collection of Type Cultures, London, United Kingdom) were used. The clinical strains were obtained from gastric biopsy specimens from patients suffering from chronic gastritis and peptic ulcer. Gastric mucosa biopsies were cultured on Columbia agar (Oxoid, Basingstoke, England) containing defibrinated horse blood 7% and *H. pylori* selective supplement (SR 147E, Oxoid). Plates were incubated microaerobically (CampyGen CNO25A, Oxoid) at 37 °C for 5-7 days. Identification was by colony morphology, Gram staining, microaerophilic growth (at 37 °C), oxidase, catalase, and urease tests (Goodwin and Armstrong, 1990).

Metronidazole susceptibility test was performed against eight *H. pylori* isolates by using the epsilometer test (Etest, AB Biodisk, Solna, Sweden). The metronidazole breakpoints of susceptible (< 8 mg/l), intermediate (= 8 mg/l,) and resistant (> 8 mg/l,) were determined according to manufacture's guide.

Stock cultures were stored at -80 °C in Brucella broth (Oxoid) containing 15% (w:v) glycerol until use.

**Inhibition of bacterial growth** Bacteria were re-cultured on Columbia agar, as described above, at 37 °C for 5-7 days. Strains were prepared in Brain Heart Infusion Broth (BHI, Merck) that contained 10% foetal bovine serum incubated 2 days under microaerobic conditions. These bacterial suspensions contained a viable count of 10<sup>8</sup> CFU ml<sup>-1</sup>. Each bacterial suspension (0,1 ml) of both clinic isolate and type strain (NCTC 11637) was inoculated on capsaicin containing microplates. DMSO control and capsaicin free control was included in microplates.

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Capsaicin (Sigma Chemical C., St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO) (Jones *et al.*, 1997). Then, different concentrations of capsaicin (25, 50, 75, 100, 150, 200, 300, and 500  $\mu\text{g ml}^{-1}$ ) were prepared and added to microplates where the bacteria were previously inoculated. The first well was free of capsaicin and the second well contained only DMSO. Different concentrations of capsaicin were put into the other wells.

Growth of bacteria in broth was assessed spectrophotometrically at 620 nm at 2 h intervals and by viable colony counts. The microplates were placed under microaerobic conditions after each measuring. To confirm if inhibition was bacteriostatic or bactericidal, after all measurements on spectrophotometer, viable bacteria counts were determined by inoculating dilutions of broth in duplicate on Columbia blood agar. Cultures were incubated for five days at 37 °C under microaerobic conditions. All the tests were repeated three times.

**Statistical analysis.** SPSS Software was performed for statistical analysis (SPSS, Chicago, IL). The analysis of variance was used to compare the concentrations and the time intervals. DUNCAN test (A Multiple range test) was used to determine the difference between the groups. A value of  $p \leq 0.01$  was considered statistically significant.

## RESULTS AND DISCUSSION

Isolates, identified as *H. pylori*, were Gram-negative, urease, catalase and oxidase positive, and showed characteristic morphology as spiral-shaped bacteria curving in microaerophilic conditions.

Concerning the inhibition of bacterial growth, the absorbance values were lower at the capsaicin-added well than the group without. Thus, we observed that capsaicin had an inhibitor effect on growth of *H. pylori* including MtzR strains. The inhibitor effect started at 25  $\mu\text{g ml}^{-1}$  and the maximal effect was found at 50  $\mu\text{g ml}^{-1}$  concentration. The same result was also evident in the experiment with the reference strain (NCTC 11637) of *H. pylori*. Incubation with only DMSO did not affect growth of bacteria.

There was no growth after inoculation of living bacteria at 2-4-6 h in plates containing capsaicin. Capsaicin killed all *H. pylori* isolates at 50  $\mu\text{g ml}^{-1}$  concentration within 4 h, however logarithmic growth was detected in plates free of capsaicin ( $2 \times 10^7$ ,  $3.5 \times 10^7$ ,  $9.0 \times 10^7$  respectively).

The viable cell counts of *H. pylori* exposed to capsaicin showed that the inhibition activity was bactericidal. There was no statistical difference between MtzR and MtzS clinical isolates regarding the bactericidal effect of capsaicin ( $p > 0.01$ ).

Furthermore, interactions between the concentrations and hours found to be significant ( $p < 0.01$ ). The effect showed variation with each time point and concentration. It has been shown that capsaicin had an inhibitor effect starting from the lowest concentration ( $p < 0.01$ ) and the maximal effect was seen at 50  $\mu\text{g ml}^{-1}$  concentration. The inhibitor effect of capsaicin at other doses is shown on graphics (Fig.1).

There are many options for the treatment of *H. pylori*. The drugs used in treatment of *H. pylori* infection have problems like undesirable side effects, contraindications, significant costs, and can result in resistance.

Metronidazole resistance among *H. pylori* strains is now

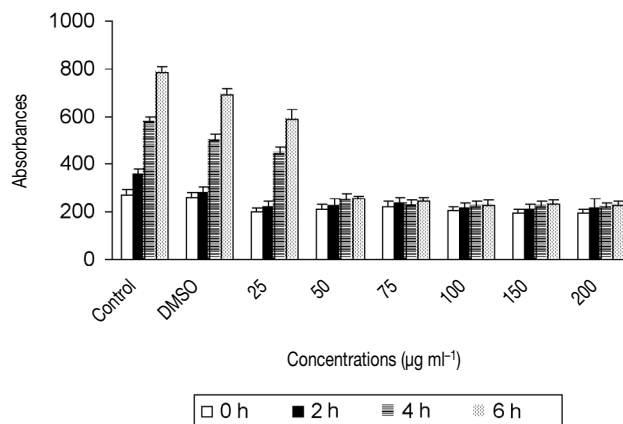


FIG. 1 – Effect of capsaicin on the growth of *Helicobacter pylori*.

found worldwide (Megraud and Doermann, 1998) with varying resistance rates (Glupczynski *et al.*, 1999). Resistance rate has been found higher in developing countries such as Turkey especially in Southeast region (Yildiz Zeyrek, 1999). Nevertheless, resistance to metronidazole remains a risk factor for treatment failure. Furthermore, the progressive increase in drug resistance warrants the need for new antibacterial drugs in the near future (Megraud, 2004).

Natural foods can be attractive as an alternative treatment of *H. pylori* (Calvet *et al.*, 2000). There are many studies supporting our hypothesis; garlic and honey have been found to be valuable in the treatment of *H. pylori* (Al Somal *et al.*, 1994; Drouin *et al.*, 1999; Mahady and Pendland, 2000).

Excessive consumption of hot pepper at Southeast part of Turkey encouraged us to perform this study. In our study, capsaicin began to inhibit both MtzR and MtzS *H. pylori* at the minimum concentration 25  $\mu\text{g ml}^{-1}$  and the maximal activity was found at 50  $\mu\text{g ml}^{-1}$  in 4 h of incubation. These results are similar to the results of a previous study reported by Jones *et al.* (1997). However, the present study has differences because of the number and types of *H. pylori* strains. Our study showed that capsaicin was also effective against MtzR clinical isolates of *H. pylori*. However, the mechanism of the antibacterial activity of capsaicin on *H. pylori* is not yet known.

Capsaicin-sensitive neurons take part in gastric mucosal defense mechanism and low doses of capsaicin has a protective effect in the rat gastric mucosa. If capsaicin were taken at 50 mg concentrations per meal, intragastric concentration would be approximately 10-50  $\mu\text{g ml}^{-1}$ , which is an anti-allergenic concentration (Holzer *et al.*, 1989; Abdel-Salam *et al.*, 1997). The amount of capsaicin is varied according to the type of pepper, and 3 to 9 peppers per person daily were enough to have efficient concentration (50  $\mu\text{g ml}^{-1}$ ) in stomach against *H. pylori* infection (Lopez Carillo *et al.*, 2003).

Capsaicin has dual effects on chemically induced carcinogenesis and mutagenesis. Although a small amount of capsaicin displays few or no deleterious effects, heavy ingestion of the compound has been associated with necrosis, ulceration and even carcinogenesis (Surh and Lee, 1996). In our study very low dose of capsaicin is used and we suggested ingestion of very little amount of capsaicin.

There is lower ulcer prevalence in people consuming

higher amount of pepper compared to controls (Kang *et al.*, 1995). It is also known that cooking can alter some chemical features of *Capsicum* species and by this way, their antibacterial effects may decrease (Cichewicz *et al.*, 1996). For this reason, it may be advisable to consume raw pepper. For people who don't like hot pepper, capsules containing capsaicin may be helpful in prevention and treatment of *H. pylori*.

In summary, the capsaicin showed an obvious *in vitro* anti-*H. pylori* activity against MtzR and MtzS clinical isolates, thus treatment with capsaicin (or hot pepper) may be a useful treatment for antibiotic-resistant strains and for patients who do not wish to take synthetic antibiotics and would also be a cheaper alternative in developing countries. Thus, it is logical to advise small amounts of hot pepper with meal to patients with gastric or duodenal ulcer. However, absolutely further animal models and clinical studies should be performed.

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